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TRPM5 Is a Voltage-Modulated and Ca^{2+}-Activated Monovalent Selective Cation Channel

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Summary

The TRPM subfamily of mammalian TRP channels displays unusually diverse activation mechanisms and selectivities [1]. One member of this subfamily, TRPM5, functions in taste receptor cells and has been reported to be activated through G protein-coupled receptors linked to phospholipase C [2, 3]. However, the specific mechanisms regulating TRPM5 have not been described. Here, we demonstrate that TRPM5 is a monovalent-specific cation channel with a 23 pS unitary conductance. TRPM5 does not display constitutive activity. Rather, it is activated by stimulation of a receptor pathway coupled to phospholipase C and by IP_3-mediated Ca^{2+} release. Gating of TRPM5 was dependent on a rise in Ca^{2+} because it was fully activated by Ca^{2+}. Unlike any previously described mammalian TRP channel, TRPM5 displayed voltage modulation and rapid activation and deactivation kinetics upon receptor stimulation. The most closely related protein, the Ca^{2+}-activated monovalent-selective cation channel TRPM4b, also showed voltage modulation, although with slower relaxation kinetics than TRPM5. Taken together, the data demonstrate that TRPM5 and TRPM4b represent the first examples of voltage-modulated, Ca^{2+}-activated, monovalent cation channels (VCAMs). The voltage modulation and rapid kinetics provide TRPM5 with an excellent set of properties for participating in signaling in taste receptors and other excitable cells.

Results and Discussion

The transient receptor potential (TRP) superfamily comprises multiple subfamilies of cation channel subunits, each of which is conserved from *C. elegans* to mammals [4]. These include the canonical or classical TRP channels (TRPC) [5, 6], which are highly related to the superfamily's founding member, *Drosophila* TRP [7], and which are activated through receptors coupled to phospholipase C (PLC) [4]. The members of the largest TRP subfamily, TRPM, display highly unusual and diverse modes of activation, selectivities, and domain organizations [1]. These members include TRPM2, TRPM6, and TRPM7, which consist of C-terminally linked enzyme domains. Another TRPM member, TRPM4, is a nonselective cation channel [8], and a long isoform, TRPM4b, is a Ca^{2+}-activated nonselective cation channel (CAN) [9] that displays properties similar to those originally described in pancreatic acinar cells and cardiac cells [10, 11].

At least two TRPM proteins function in sensory physiology. TRPM8 is activated by cool temperatures [12, 13], and TRPM5, which is enriched in taste receptor cells, is required for the taste modality [2, 3]. TRPM5 has been reported to conduct divalent cations and to be activated through a G protein-coupled receptor/PLC signaling pathway [2, 3]. According to one report, TRPM5 is activated through a store-operated mechanism [2], although a second study concludes that TRPM5 activation is independent of Ca^{2+}, IP_3, and store depletion [3]. Thus, the mechanism of TRPM5 activation is unclear and is the focus of the current report.

In addition to taste receptor cells, TRPM5 RNA is detected in a variety of tissues, including the small intestines, liver, lungs, testis, and brain. To isolate mouse TRPM5 cDNAs, we performed RT-PCR by using RNA prepared from pooled mouse lung and brain tissue. We obtained a cDNA encoding a predicted protein of 1158 amino acids, whose sequence was identical to the previously reported sequence [14].

To characterize the activation mechanism, we coexpressed TRPM5 in 293T cells with GFP and a G protein-coupled receptor (H_1 histamine receptor), which was coupled to the activation of PLC. Control transfected cells stimulated with histamine did not display any currents (Figure 1A, upper trace; n = 0/12). However, application of histamine to TRPM5-expressing cells induced rapidly activating currents, which quickly decayed to baseline (Figure 1A, lower trace; n = 13/13; mean amplitude of −1.52 ± 0.43 nA). In the presence of 100 μM La^{3+}, somewhat smaller histamine-induced currents were detected (−638 ± 357 pA, n = 5), but these had normal kinetics (data not shown). The rapid time course of the transient TRPM5 current was also observed in single-channel recordings in the in cell-attached mode (Figure 1C; n = 6). No channel events were observed in control cells that did not express TRPM5 (data not shown). TRPM5 was also activated upon addition of ATP, which stimulated the endogenous P2Y receptor, although the amplitude was smaller than with histamine (Figure S1 in the Supplemental Data available with this article online), presumably due to higher expression of the transfected H_1 histamine receptor.

In order to determine precisely the onset, activation, and inactivation kinetics of TRPM5, we applied the stimulus through a puffer pipet in the immediate vicinity of the cell. Under these conditions, the mean time to onset of the currents was 590 ± 55.7 ms (Figure 1B), a latency similar to that previously reported for acetylcholine-induced currents in pancreatic acinar cells [15]. The time-course of these transient currents could be fit by

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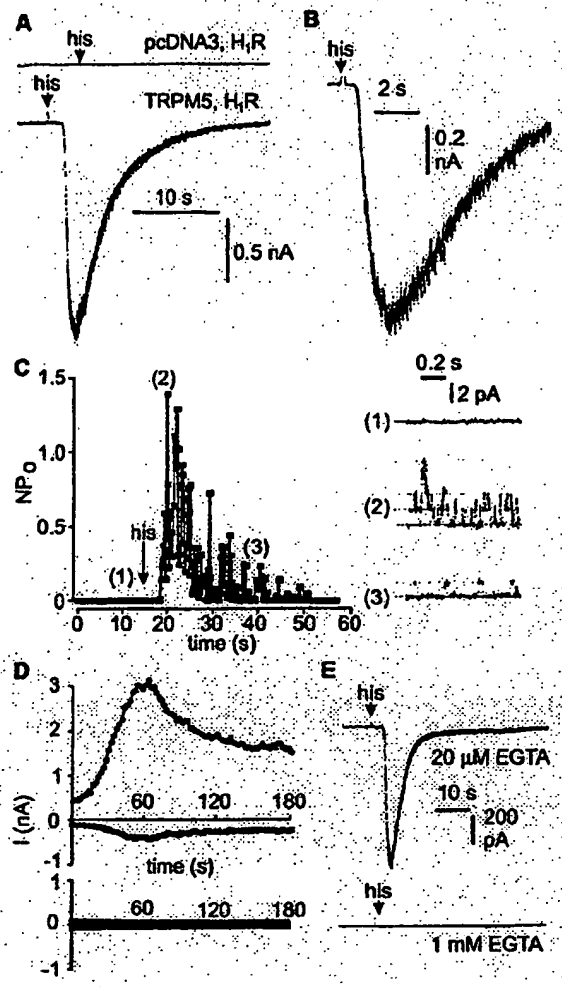

Figure 1. Receptor-Mediated Activation of Mouse TRPM5

(A) Induction of a transient histamine-induced current in TRPM5-expressing 293T cells. A cell expressing TRPM5 and the H_1 histamine receptor (H_1R, lower trace) and a cell containing the empty vector, pcDNA3, and the H_1R (upper trace) were stimulated with 100 μM histamine (his) at the time indicated. Shown are the whole-cell recordings generated from the TRPM5-expressing and control cells (V_h = −60 mV).

(B) The TRPM5-dependent current is rapidly induced by histamine. Shown is a whole-cell TRPM5 transient current generated by application of histamine (100 μM) through a puffer pipet. Note the different timescales in panels (A) and (B). A biexponential pulse function was fit to the data.

(C) Single-channel recordings in the cell-attached mode (V_h = 60 mV) generated from TRPM5-expressing cells stimulated with 100 μM histamine. The left panel displays the time-course of the apparent open probabilities (NP_o). The single traces shown to the right were obtained from the time points indicated in the NP_o panel.

(D) Time-course of the whole-cell current after a TRPM5-expressing 293T cell was perfused with 10 μM IP_3. Ramp currents were applied at 2 s intervals (V_h = 60 [dots] or −60 mV [squares]). EGTA (20 μM) was included in the pipet solution. The lower panel shows a TRPM5-expressing 293T cell stimulated with 10 μM IP_3, except that $[Ca^{2+}]_i$ was buffered to 300 nM with 10 mM BAPTA.

(E) Histamine-induced transient whole-cell current in a TRPM5-expressing 293T cell (V_h = −60 mV). The cell was perfused with standard pipet solution containing 20 μM EGTA. The histamine (100 μM) was added at the time indicated. The lower panel shows that addition of 1 mM EGTA to the recording pipet suppressed the histamine-induced TRPM5 response.

the product of two exponentials, yielding an activation time constant of 960 ± 73 ms. The rapid inactivation/deactivation of the inward current had a time constant of 2960 ± 340 ms (n = 5).

Given that TRPM5 was activated by a receptor coupled to Gαq, we investigated the activation mechanism downstream of PLC. We found that inclusion of 10 μM IP_3 in the recording pipet activated TRPM5 in the absence of receptor stimulation (Figure 1D, upper panel; n = 5; −1.22 ± 0.42 nA). We obtained similar results by perfusing the cells with 100 nM adenophostin A, a potent activator of IP_3 receptors (data not shown). When we strongly buffered intracellular free Ca^{2+} (10 mM BAPTA, 300 nM free-Ca^{2+} concentration), the IP_3-mediated activation of TRPM5 was abolished (Figure 1D, lower panel; n = 3). The rapid histamine-induced activation of TRPM5 was blocked by the IP_3-receptor inhibitor, heparin, although after a long delay the cells displayed large, slowly activating and slowly inactivating currents (data not shown). Addition of a SERCA inhibitor (thapsigargin) resulted in a transient response, which was much smaller than that generated with IP_3, indicating that passive release of Ca^{2+} was inefficient in activating TRPM5 (Supplemental Figure 2). Together these data indicate that IP_3-mediated Ca^{2+} release is critical for the activation of the rapid but transient receptor-mediated TRPM5-dependent current.

The observation that IP_3 receptor-mediated Ca^{2+} release is required for activation of TRPM5 raises the question as to whether Ca^{2+} is the exclusive mechanism through which TRPM5 is activated. Addition of a Ca^{2+} ionophore (1 μM ionomycin) to TRPM5-expressing cells bathed in 2 mM $CaCl_2$ resulted in large, transient TRPM5 currents (Figure 2A; n = 3; −960 ± 358 pA). The onset and amplitude of the TRPM5 currents were dependent on the free-Ca^{2+} concentration in the pipet solution in the absence of any receptor stimulation (Figures 2B and 2C). These results indicate that Ca^{2+} is sufficient to activate TRPM5.

The preceding results suggest that Ca^{2+} is the crucial mediator of receptor-induced TRPM5 activation. To test this proposal directly, we chelated intracellular Ca^{2+} with EGTA (1 mM) and performed whole-cell recordings after stimulating the 293T cells with histamine. Control cells perfused with the standard pipet solution displayed normal histamine-activated currents (Figure 1E; n = 3). However, perfusing the cells with EGTA prior to addition of histamine precluded receptor-mediated channel activation (Figure 1E; n = 3). These data indicate that Ca^{2+} is the key mediator of receptor-dependent activation of TRPM5.

Several TRP channels bind to calmodulin [16], raising the question as to whether activation of TRPM5 by Ca^{2+} occurs through calmodulin. However, receptor stimulation of TRPM5 was not inhibited by perfusion of the cells with a MLCK calmodulin-inhibiting peptide (10 nM) prior to stimulation with 100 μM histamine (Figure S3A; n = 3). Addition of another calmodulin inhibitor, calmidazolium,

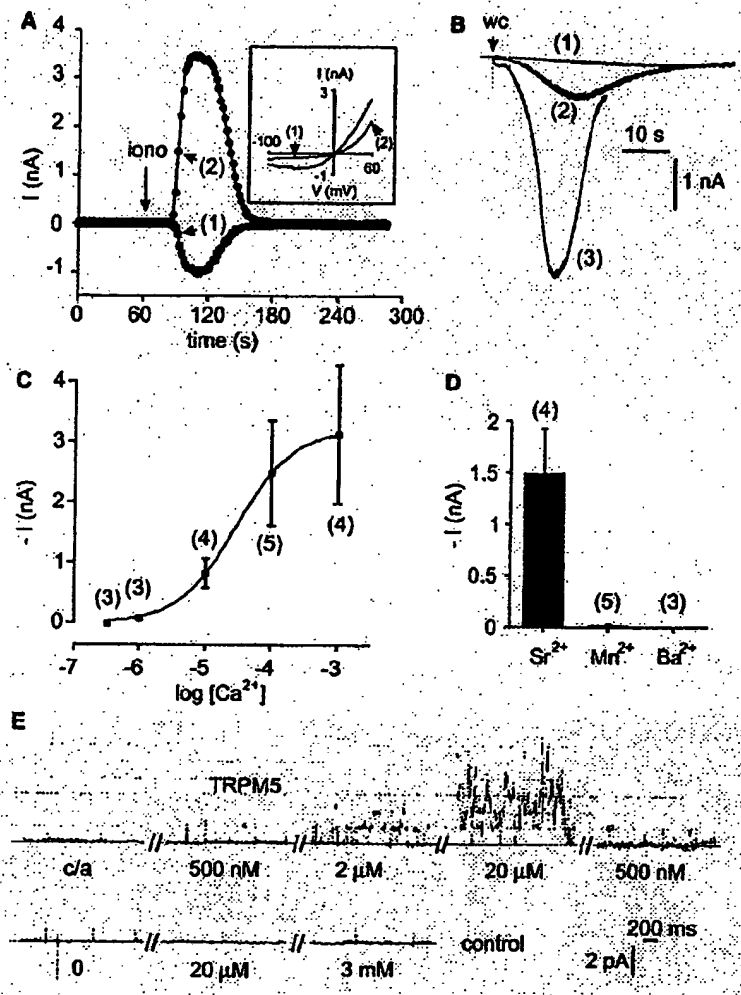

Figure 2. TRPM5 Is Activated by Elevations in $[Ca^{2+}]_i$

(A) Whole-cell recording of a TRPM5-expressing cell stimulated with 1 µM ionomycin as indicated. The inset shows voltage ramps applied starting at holding potentials of (1) −60 mV or (2) 60 mV. The time-courses of the currents were extracted from the ramps at the points indicated.

(B) Time-course of the whole-cell currents obtained from representative cells infused with a pipet solution containing a free-Ca^{2+} concentration of (1) 1 µM, (2) 10 µM, or (3) 100 µM (V_h = −80 mV). The whole-cell configuration (wc) was established at the indicated time-point.

(C) Concentration-response relationship of the TRPM5 whole-cell currents at −80 mV. The data were fit with a Hill equation, yielding an EC_{50} of 30 µM. The numbers of independent experiments are indicated in brackets.

(D) Summary of the whole-cell current amplitudes during infusion of the cells with 300 µM Sr^{2+}, 300 µM Mn^{2+}, or 10 mM Ba^{2+} (V_h = −60 mV).

(E) Effects of Ca^{2+} on the single-channel currents. The upper panel shows sample currents obtained from a TRPM5-expressing cell, starting in a cell-attached configuration (c/a). The same patch was subsequently excised into a bath containing 500 nM free Ca^{2+} and then exposed to the indicated concentrations of free Ca^{2+}. The lower panel shows the absence of current in an excised patch taken from a control cell, which does not express TRPM5. All of the experiments were carried out at a V_h of 60 mV. The solid and dotted lines reflect the open or closed states, respectively.

may have had a minor effect on TRPM5 activation, but it was not statistically significant (Supplemental Figure 3B; n = 8). Moreover, at very high concentrations of Ba^{2+}, which have been reported to facilitate activation of calmodulin (10 mM) [17], no TRPM5 current was observed (Figure 2D).

The observation that activation of TRPM5 does not require calmodulin raises the possibility that activation by Ca^{2+} may be direct. Such a proposal was supported by data obtained from inside-out patches excised from TRPM5-expressing cells. In the cell-attached configuration, we did not observe single channel activity (Figure 2E; n = 3). However, after excision and subsequent introduction of a Ca^{2+}-containing solution, we observed rapidly appearing and concentration-dependent single channel activity, which was reversible (Figure 2E). Each patch typically contained numerous channels, resulting in macroscopic currents of several tens to more than 100 pA at saturating Ca^{2+} concentrations (>100 µM). Single-channel events were observed also at low µM concentrations of Ca^{2+}, with a threshold of 500 nM. In inside-out patches excised from control transfected cells, we did not observe channel events, even at millimolar concentrations (Figure 2E, lower panel; n = 6). Thus, it appears that TRPM5 is activated either directly by Ca^{2+} or through a protein other than calmodulin that is closely associated with the channel.

To determine the ion selectivity of TRPM5, we assessed the relative shifts in the reversal potential after exchanging the bath solution with different cations (Figure 3A). The recordings were performed in the whole-cell mode with a NaCl-based pipet solution, which contained 10 µM free Ca^{2+}. There was little change in the reversal potential (which was close to 0 mV) after the extracellular solution was exchanged with 140 mM KCl or 140 mM CsCl. However, replacement of the extracellular cations with 140 mM NMDG, 100 mM $MgCl_2$, or 100 mM $CaCl_2$ resulted in pronounced inhibition of the inward component of the current and a shift of Vrev toward −60, −50, or −53.3 mV, respectively. Based on these results, the relative permeabilities of K^+, Cs^+, $NMDG^+$, Mg^{2+}, and Ca^{2+} versus Na^+ were 1.0, 1.0, 0.1, 0.06, and 0.05, respectively.

In order to determine if TRPM5 could permeate any appreciable concentration of divalent cations, we performed further analysis on excised inside-out patches,

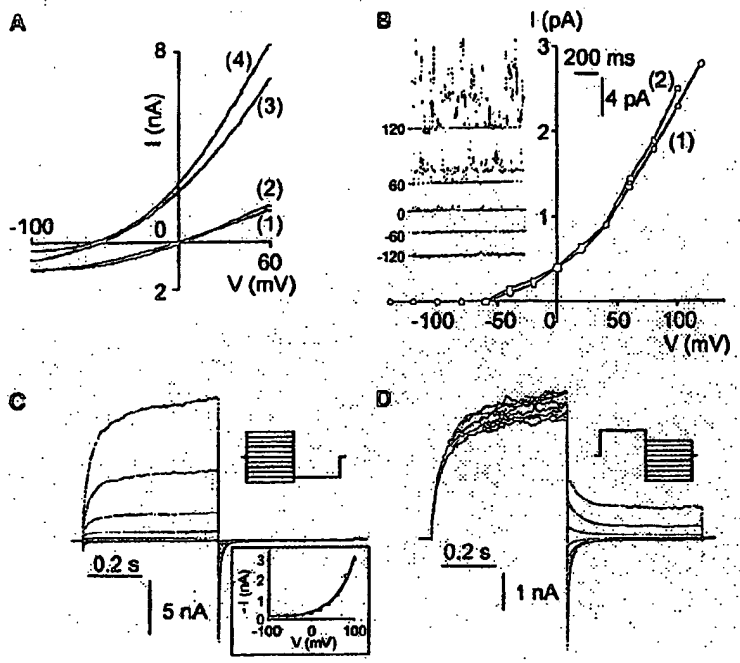

Figure 3. TRPM5 Is a Voltage-Modulated Monovalent-Selective Cation Channel

(A) TRPM5-expressing cells were perfused in the whole-cell mode with activating concentrations of Ca^{2+}. Voltage ramps were applied from 60 mV to −100 mV after exchange of the standard bath solution with solutions containing (1) 140 mM NaCl, (2) 140 mM KCl, (3) 100 mM $CaCl_2$, or (4) 140 mM NMDG-Cl.

(B) Current-voltage relationship of TRPM5 excised patches in a bath solution containing 140 mM NaCl and 100 μM Ca^{2+}. The pipet solutions contained either (1) 100 mM $CaCl_2$ or (2) 100 mM $MgCl_2$. The inset shows sample traces from an excised patch in 100 mM $CaCl_2$ at 120, 60, 0, −60, and −120 mV.

(C and D) TRPM5-expressing cells were perfused with 1 μM free Ca^{2+} and subjected to the voltage paradigms shown in the insets. (C) The cell was held at 0 mV, challenged with voltage steps from −100 to 100 mV, and stepped to −80 mV. The inset shows the tail current-voltage relationship, which was extracted from the experiment in (C), fit by an exponential. (D) The cell was stepped from 0 to 100 mV and exposed to different test potentials ranging from −100 to 60 mV.

which were exposed to 100 μM Ca^{2+} in a Na^+-based bath solution. In the presence of either $CaCl_2$ or $MgCl_2$ as the main cations in the pipet solution, the outward currents were clearly detectable, but no inward currents were observed at negative holding potentials (Figure 3B; inset shows an experiment with 100 mM $CaCl_2$). The average single channel conductance in these experiments (at 60 mV) was 23 pS, which was in agreement with the slope conductance of 23 pS that we found in separate experiments analyzing single-channels in the cell-attached mode (data not shown). Thus, TRPM5 is a 23 pS, monovalent-selective cation channel that poorly discriminates between monovalent cations but precludes divalent cations.

Consistent with most TRP proteins, we found that the open probability of the TRPM5 current showed a voltage dependence, which results in strong outward rectification. However, in contrast to other reported TRP-dependent conductances, I_{TRPM5} displayed a slow current relaxation after voltage steps in either direction (Figure 3C). In addition, I_{TRPM5} was associated with pronounced tail currents when the voltage stepped to −80 mV from a positive potential. The steady-state activation, as determined by analysis of the amplitude of the tail currents, was steeply voltage dependent and best fit by an exponential curve (Figure 3C, inset). In addition, the tail currents after a step to the same potential were slightly slower at more positive potentials (Figure 3D).

The TRPM protein that shares the greatest sequence identity with TRPM5 is TRPM4 (45%; Figure 4A). Human TRPM4 is expressed as two isoforms, one of which, TRPM4b, is a CAN [9]. However, expression of TRPM4a results in small currents that are not consistently observed (Figure 4C, inset, n = 4/9). Therefore, we introduced TRPM4b in 293T cells and addressed whether it displays voltage modulation similar to that of TRPM5. We found that the TRPM4b currents showed voltage modulation (Figure 4B), although they had slower relaxation kinetics than TRPM5 currents. As with TRPM5, the TRPM4b tail currents were best fit by the sum of two exponentials. The slower predominant exponential had a time constant of 9.1 ± 2.1 ms (n = 6) and 28.0 ± 2.4 ms (n = 6) for TRPM5 and TRPM4b, respectively. Thus, although both channels were voltage modulated, the voltage relaxation kinetics was approximately 3-fold slower for TRPM4b. Also similar to TRPM5, we found that TRPM4b displayed a rapid transient response to receptor stimulation (Figure 4C). Based on the activation and permeation properties of TRPM5, as well as TRPM4b, we refer to them as a voltage-modulated and Ca^{2+}-activated monovalent-specific cation channels (VCAMs). This set of features, combined with the rapid kinetics, is distinct from that reported for other TRP channels.

Ca^{2+}-activated monovalent cation currents (CAMs) are widespread and have been described in nonexcitable cells as well as in excitable cells such as myocytes, sensory cells, and neurons in the central nervous system [18, 19]. CAMs have been implicated in such diverse phenomena as the slow oscillations in the thalamocortical neurons during sleep [20], the firing properties of pyramidal cells in the cortex [21], and signaling in cardiomyocytes [22, 23]. Despite extensive analyses of CAMs, the channels responsible for these conductances have been elusive.

TRPM4b [9] and TRPM5 share many of the hallmarks of CAMs. These include single-channel conductances between 20 and 35 pS, Ca^{2+} activation, selectivity for monovalent cations, and low permeability to Ca^{2+}. Other than TRPM4b and TRPM5, there are no other channels described with these features. Thus, these two TRP channels are currently the only molecular candidates

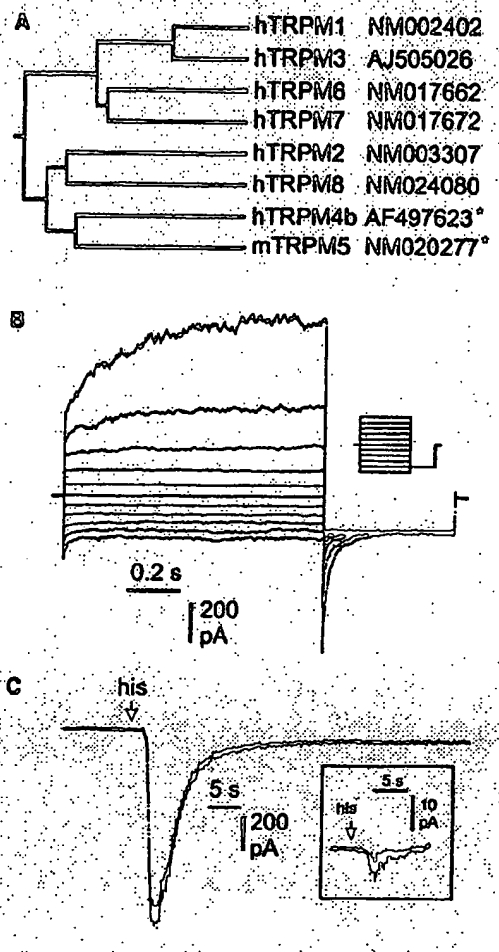

Figure 4. Human TRPM4b is a VCAM Similar to TRPM5

(A) A TRPM subfamily phylogenetic tree calculated with the Clustal algorithm. The GenBank accession numbers are indicated. The accession numbers for hTRPM4b and mTRPM5 obtained in the current report are AY297045 and AY280364, respectively.

(B) TRPM4b was voltage-modulated. A TRPM4b-expressing cell was preactivated by infusion with 10 μM Ca^{2+}. A whole-cell voltage-step paradigm, which was similar to that in Figure 3C (inset), was applied.

(C) Activation of TRPM4b by histamine is rapid and transient. Receptor-activated whole-cell current in a TRPM4b-expressing cell (V_h = −60 mV). Histamine (100 μM) was applied at the time indicated. The inset depicts a representative cell expressing human TRPM4a.

that could account for CAMs. Such TRP channels provide a mechanism for cells to sense and respond to increases in Ca^{2+} without directly changing the intracellular levels of Ca^{2+}. However, CAMs might modulate the magnitude of Ca^{2+} influx indirectly through effects on the membrane potential [9, 24].

The finding that TRPM5 and TRPM4b are rapidly activated and inactivated voltage-modulated CAMs raises the possibility that many of the endogenous CAMs, which have been described in excitable cells, are actually VCAMs. The previous studies typically do not include analyses of current/voltage relationships at a variety of potentials; therefore, the voltage regulation would have eluded detection. Although the slow current relaxation following voltage steps and the fast transient response to agonist stimulation have not been previously reported for TRP channels, such features are hallmarks of Ca^{2+}-activated Cl^- channels [25].

Because TRPM5 is widely expressed, it would be interesting to address whether the TRPM5 knockout mice [3] exhibit cardiac arrhythmias or affect sleep rhythms, cortical function, or other processes suggested to depend on CAMs. Furthermore, it would be worth reinvestigating the currents in wild-type and *TRPM5*$^{-/-}$ taste receptor cells by examining the current/voltage relationships and other properties of TRPM5 described in the present report. The features of TRPM5 outlined here seem to make it well suited for responding to changes in membrane potential and fluxes in Ca^{2+}, as occurs during sensory transduction. These include the high sensitivity to receptor stimulation, voltage-regulation, the virtual absence of noise because there is no constitutive activity, the ability to sense a rise in Ca^{2+} without conducting Ca^{2+}, and the rapid kinetics of activation and deactivation.

Supplemental Data

Supplemental Experimental Procedures as well as three additional figures are available with this article online at http://www.current-biology.com/content/supplemental.

Acknowledgments

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Accession Numbers

We report the following accession numbers in this paper: TRPM4b cDNA, AY297045; and TRPM5 cDNA, AY280364.